THE CELLULAR ELEMENTS IN THE PERIVISCERAL FLUID OF ECHINODERMS.

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A consideration of the cellular elements in an animal group, particularly those cellular elements which are present in cavities derived from the primitive cœlom, should include references to the origin of these cells and any other factors which may be concerned with their modifications, such as the habits, the powers of regeneration and the topographical anatomy of the organ systems of the animals examined. It is generally conceded from the evidences of palæontology and comparative embryology that the Echinoderms of to-day are a fairly ancient group which have been able to adapt themselves to diverse environmental changes. The same pentaradial symmetry is present in all of them, although masked in some, but the principal variations which are characteristic of the classes are concerned primarily with the character of the body wall and secondarily with the distribution of the breathing organs. In the Echinoderms we can distinguish three types of organization of the body wall and breathing organs: the first type is characterized by a fairly flexible body and diffuse breathing organs (*e.g.*, Asteroidea and Ophiuroidea), the second by a rigid test and limited breathing organs (*e.g.*, Echinoidea), and the third by a well-developed muscular body wall and limited breathing organs (*e.g.*, Holothuroidea). In all of the classes except the Holothuroidea, the movements of the body are very sluggish, consequently the oxygen requirements for muscular activity are very low and a system for rapid transfer of oxygen is not needed. But in the majority of the Holothuroidea, the development of muscle necessitates a great available supply of free oxygen and a mechanism for carrying this oxygen must be present. Therefore one phase of this investigation will attempt to correlate the appearance of different types of cells in the perivisceral fluid with the character of the body wall, the distribution of the breathing organs and the movements of the body as a whole.

We know that the Echinoderms have great powers of regeneration and may conclude, therefore, that all of the cells in the body are very labile, but the question arises as to which cells in the perivisceral fluid are the most generalized. Cells which could be regarded as the most generalized would be those which are constant in the perivisceral fluid of all of the Echinoderms and which are observed to have diversified functions; those cells which under necessity of local needs remove foreign material, wornout fragments of cellular origin and which could give rise to modified cells. Another phase of this investigation, then, is to determine if there are any such cells in the perivisceral fluid, and if so, what is the nature of their activities.

I. Material and Methods.

The material used in this investigation was collected in the vicinity of the Puget Sound Biological Station, Friday Harbor, Washington. Representatives of the four classes of Echinoderms found in this area were used. The following are the species which have been examined:

Class I. Asteroidea.

1. *Evasterias troschelii.*
2. *Solaster simpsonii.*
3. *Dermaster imbricata.*
4. *Pisaster ochraceus.*
5. *Leptasterias hexactis.*
6. *Henricia leviuscula.*
7. *Pycnopodia helianthoides.*

Class II. Ophiuroidea.

1. *Ophiopholis aculeata.*

Class III. Echinoidea.

1. *Strongylocentrotus drobachiensis.*
2. *Strongylocentrotus franciscanus.*
3. *Echinarachnius eccentricus.*

Class IV. Holothuroidea.

1. *Cucumaria japonica.*
2. *Cucumaria chronjhelmii.*
3. *Stichopus californicus.*

Perivisceral fluid from individuals of each of the above species was drawn from the perivisceral cavity and studied by the hanging drop method. In studying the phagocytic activity of the cells, a concentrated suspension of finely granulate carmine or india ink in seawater was used. This suspension was injected into the perivisceral cavity through a minute opening in the body wall by means of a delicate hypodermic needle. The amount of suspension injected varied with the size of the animal, but it was found that the usual dose sufficient to affect the phagocytes was 8 cc. The injected animals were kept in a live box for a day, so that there would be time for thorough ingestion of the particles.

The clotting activities of the cellular elements were studied in drops of perivisceral fluid which had been allowed to stand for varied lengths of time.

A saturated solution of seawater and methylene blue, another of seawater and neutral red were made and allowed to stand for several days before using. The supernatant solution which was free from particulate matter was decanted off and the solution used drop for drop with the perivisceral fluid. These stains were used for intravitam staining in certain phases of the investigation since they were found to be specific for the vacuoles of the leucocytes.

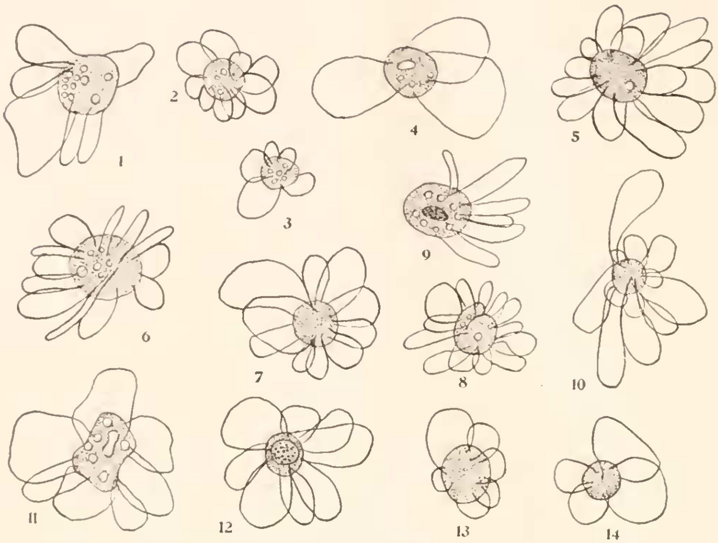
II. Observations.

1. *The Leucocytes.*

In his paper on the leucocytes of the invertebrates, Goodrich ('19) called attention to the fact that the leucocytes of *Asteracanthion glacialis* are characterized by the presence of extensive membranous processes of the ectoplasm. He says, "The freely projecting pseudopodia usually described are either figured from optical sections of the folded membranes or from cells which have produced them under abnormal conditions. These pseudopodia may be present on cells in the fluid withdrawn from the body and which has been allowed to stand, and are probably derived from preëxisting membranes." Goodrich calls all of the cells leucocytes, making no distinctions.

In *Arbacia* (Kindred, '21) I observed the formation of a syncytium in the perivisceral fluid by the anastomosis of filiform processes which had been derived from the membranous flaps of the leucocytes, thus supporting Goodrich's assumption. Théel ('21), however, in a review of his descriptions of the types of amœbocytes in the perivisceral fluid of *Asterias rubens* and *Parechinus miliaris*, states that he distinguished two types of amœbocytes in the cœlomic cavity of these forms, the "white or hyaline plasma-amœbocytes" and the "bladder amœbocytes." These descriptions were not referred to by Goodrich nor myself although they appeared in Swedish prior to both of our papers. According to Théel the two types of amœbocytes are very dissimilar, the "bladder amœbocytes" not being flattened nor spread out on the surface of the glass when a drop of the fluid is placed on a glass slide, but remaining thick and compact with the bladder lying in several superimposed layers. On the other hand, the "hyaline plasma-amœbocytes" in the fresh fluid are characterized by the possession of a concentrated cell body with longer or shorter pseudopodia. Théel expresses doubt as to whether or not one type may be derived from the other. Following the terminology used by Goodrich, I am calling the cells with the membranous flaps leucocytes, and regarding Théel's "hyaline plasma-amœbocytes" as a secondary phase of the leucocytes from my observations on the formation of syncytia. Théel ('21) in expressing an opinion as to the possibility of morphological changes in such cells says: "However, it may be presumed that the character of the surrounding medium may play an important part in that, and above all movement and relative stillness, the former preventing and the latter forwarding the process of transmutation. If for instance an amœbocyte leaves the cœlomic cavity in order to immigrate into the tissues of the body wall, it must necessarily undergo certain changes of form. When a cell passes over from a passive drift to an active motion, its primitive globular configuration must be exchanged for another and accommodated to creeping about."

Therefore, the presence of two forms of cells in the freshly drawn drop does not of necessity mean that because of this occurrence we are dealing with two distinct types instead of an active and passive form of one type of cell. It is reasonable to suppose that these cells have a cycle of life and that as they become older this change to a more passive condition leads to a change in form. Since the activities of these cells *are* comparable to the activities of the leucocytes in other animals, they are so termed.



FIGS. 1–14. Active leucocytes of the Echinoderms, camera lucida, × 650. 1. *Leptasterias hexactis.* 2. *Solaster simpsonii.* 3. *Dermaster imbricata.* 4. *Pycnopodia helianthöides.* 5. *Pisaster ochraceus.* 6. *Henricia leviuscula.* 7. *Evasterias troschelii.* 8. *Strongylocentrotus drobachiensis.* 9. *Ophiopholis aculeata.* 10. *S. franciscanus.* 11. *Echinarachnius eccentricus.* 12. *Cucumaria japonica.* 13. *C. chronjhelmii.* 14. *Stichopus californicus.*

Text-figures 1–14 are camera lucida drawings of the active phases of the leucocytes ("bladder amœbocytes" of Théel) of the whole series of Echinoderms studied. Examination of these figures shows the general morphological similarity of these cells to each other, the only distinct difference being that of size, which varies from 7–14 microns in endoplasmic diameter. In all of the cells it will be noted that the ectoplasm is clearly marked off from the endoplasm and is produced into a varying number of rapidly changing delicate flaps. These flaps are constantly being withdrawn and extended and may be regarded as modified pseudopodia. By means of these flaps the leucocyte progresses slowly through the fluid. That the surface of the cell is covered with a sticky fluid is evidenced by the manner in which particulate matter adheres to the flaps. When the flaps are withdrawn the particles which adhere to them are ingested.

The endoplasm of nearly all of the active leucocytes is granular and opaque, the exceptions to this opacity are found in the leucocytes of *Ophiopholis aculeata* (Fig. 9) and *Cucumaria japonica* (Fig. 12). In these leucocytes the nucleus with its content of large chromatin granules is easily discernible. The endoplasm usually contains a varying number of hyaline vacuoles which stain blue with the methylene blue-seawater solution and red with the neutral red-seawater solution.

The active leucocyte is a phagocyte and is always found loaded with carmine or india ink particles when these substances are introduced into the perivisceral cavity. In the Holothuroidea these phagocytes were observed to deposit the india ink particles in the skin (Schultz, '95). Awerinzew ('11) considers that the color of the test in some of the Echinoidea is due to this phagocytic activity of "amœbocytes" and that the color difference in varieties of *S. drobachiensis* is dependent upon the color of the food, the pigments of which are taken up by the "amœbocytes" and carried to the skin. Since the leucocytes are the only types of cells in the perivisceral fluid which are phagocytic, they are without doubt the phagocytic "amœbocytes" whose activities are described by these and other authors. A further function of the phagocytes is the removal of germ cells remaining in the gonads after the bulk of the gametes have been shed (Caullery and Siedlicki, '03). Cernovodeanu and Henri ('06) observed that bacteria injected into the body cavity of sea urchins were taken up by "amœbocytes" with long pseudopodia, cells which are doubtless leucocytes. Since mention has been made above of the relation of the leucocytes to the transfer of food products, it is pertinent to enquire as to further observations of their participation in this phase of vital activity.

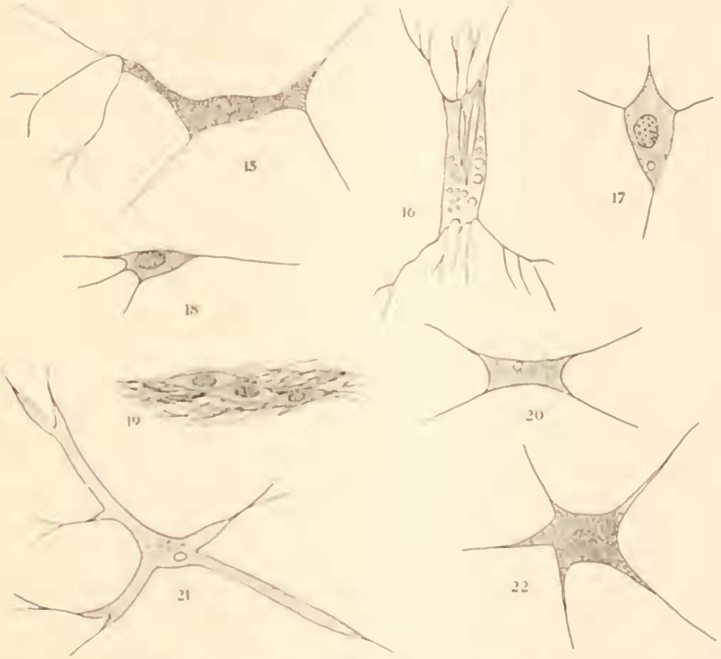
Cuenot ('91b), one of the first to call attention to this relation, assumed that the substances passed from the intestinal cells into the intestinal lacunæ are taken up by the "amœbocytes" and stored up in them to be carried to other parts of the body. The "amœbocytes" which are so concerned become metamorphosed into "amœbocytes with spherules." Frenzel ('92) was of the opinion that the "amœbocytes" pushed between the epithelial cells of the intestine and into its lumen where they disintegrated and their remnants acted as a digestive ferment. Enriques ('02) thought that digestive substances were carried from the rete mirabile peritoneum in the Holothuroidea to the stomach epithelium by "amœbocytes." Thus there are several conceptions concerning the relations of the leucocytes to the digestive activities which need further investigation.

As evidenced by their tendency to remove foreign particles from the body cavity the leucocytes may be regarded as excretory agents and further observations as to their excretory activities should be considered. Durham ('88) and Chapeaux ('93) observed that the phagocytic cells leave the body through the papulæ in the Asteroidea. I have observed such a migration in *Leptasterias hexactis*, in which, after injection with carmine, the papulæ are reddish and a smear from the outer surface reveals a number of leucocytes laden with carmine particles. "Amœbocytes" (particular type not stated) have been observed to leave the body cavity of the Holothuroidea by diapedesis through the walls of the branchial tree into its lumen and thence to the outside (Herouard, '95; Schultz, '95). Therefore, there is evidence that the exit of the phagocytes in the Echinoderms is through the body wall.

The exact relation of the leucocytes in the removal of waste substances from the tissues has not been proven, but Delage and Herouard ('03) thought that substances absorbed from the tissues by "amœbocytes" are reprecipitated in them in the form of granules and may possibly give rise to the various "amœbocytes with spherules." List ('97) pointed out earlier that substances absorbed by the "amœbocytes" may be the cause of the development of a crystalloid in the nucleus of cells of this type, which by growth causes a degeneration and finally the destruction of the cell. Thus the accumulations of crystalloids observed scattered throughout the body of various Echinoderms may be regarded as the remnants of degenerate excretory "amœbocytes."

Another activity of the active leucocytes is the formation of plasmodial masses which are very numerous in any drop of perivisceral fluid. That these plasmodia are formed by the fusion of active leycocytes has been observed frequently and plasmodial formation is one of the activities which distinguishes the active from the passive phase of the leucocyte and from other cells in the perivisceral fluid.

In addition to the active leucocytes there are always present, even in a drop of freshly drawn perivisceral fluid, numbers of flattened cells with filiform processes which float passively through the fluid (Figs. 15–22). The endoplasm of these cells is granulated, opaque and vacuolated, as is the endoplasm of the active leucocytes and it reacts the same to methylene blue-seawater or neutral red-seawater solutions, *i.e.*, the vacuoles stain blue and red respectively. Since I have observed active leuco-

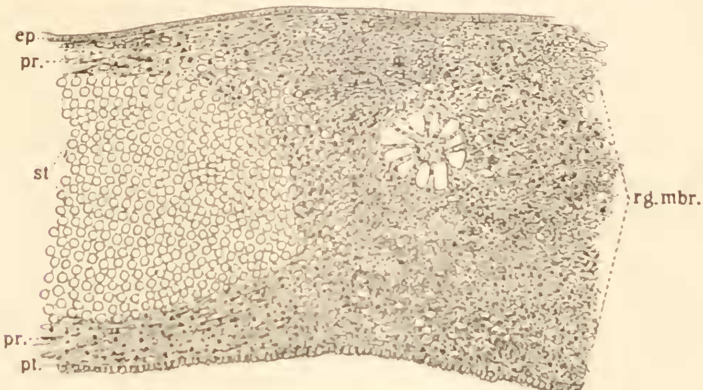


FIGS. 15–22. Passive leucocytes of the Echinoderms, camera lucida. × 650. 15. *Dermaster imbricata.* 16. *Henricia leviuscula.* 17. *Cucumaria japonica.* 18. *Stichopus californicus.* 19. *Strongylocentrotus drobachiensis.* 20. *Solaster simpsonii.* 21. *Pisaster ochraceus.* 22. *Ophiopholis aculeata.*

cytes changing into these in *Arbacia*, I think that they are passive phases of the leucocytes. Théel regards them as distinct in themselves and calls them "hyaline plasma-amœbocytes." Their presence in the freshly drawn fluid would indicate that they are normally present and that their occurrence is not due to abnormal conditions as Goodrich suggested. Although these cells may occur singly, they are most frequently met with as elements of a syncytium and it is this syncytial formation which is their most valuable vital activity, in that it is concerned with the repair of injury and the replacement of lost parts in the organism. This is due to the fact that these syncytia close wounds and form the basis for the growth of the other tissues and further that these cells themselves may take part in the formation of other tissues.

Théel ('21) states that there is a fibrin-like substance which occurs in the coagulation of the cœlomic fluid in the Holothuroidea in addition to the meshwork of the fibers formed by the arborizing leucocytes. That this fibrin-like substance is extracellular is evidenced by the fact that a fibrous meshwork occurs when there are far too few cells to form such a meshwork so rapidly, by leucocytic syncytia alone. I observed this superfluity of fibers in the drops of perivisceral fluid of all of the preparations of fresh material and was at a loss to account for it. The origin of this fibrous substance is not known, although it has been suggested by Schäfer ('83) that it might have been secreted by the leucocytes. That it does not coagulate in the fluid in the perivisceral cavity is due to the various ciliated cells which keep the fluid in motion (Cuenot, '01). This movement of the perivisceral fluid does not prevent the formation of plasmodia or syncytia, but it does seem to inhibit the fibrin-like coagulum.

From the observations of Théel and others it is well known that in the development of the test of the Echinoidea the leucocytes form syncytia within which is secreted (intracellularly) the spicules which fuse and form the stereom, a definite beam and rafter skeletal structure. Leucocytes containing spicules have been observed in the perivisceral fluid of various Echinoderms. I have observed them in the perivisceral fluid of *Henricia leviuscula* (Fig. 16). Théel from his observations on the occurrence of these cells containing spicules is of the opinion that we may presume that the skeleton of the Echinoderms is due to the activity of migratory "plasma-amœbocytes" and their syncytial fusion. This assumption may be true, but there has been no evidence presented which shows that these cells (my leucocytes) are concerned in the replacement of resected areas of the stereom. Hence in order to determine the scleroblastic activity of the leucocytes a series of resections of the test of *S. drobachiensis* were made and the subsequent regeneration observed. But before discussing the observations the relationships of the stereom and the adjacent region should be described. Thus in a section



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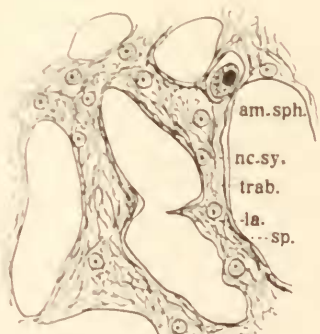

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FIG. 23. Regenerating body wall, *Strongylocentrotus drobachiensis*, vertical section, semi-diagrammatic, camera lucida, × 75.

FIG. 24. Detail of reticulum of regenerated membrane of body wall shown in Fig. 23. Semi-diagrammatic, camera lucida, × 650.

Fig. 25. Detail of prestereomal area shown in Fig. 23. Semi-diagrammatic, camera lucida, × 650. *am.sph.*, "amœbocyte with spherules"; *ep.*, epidermis; *la.*, lacuna; *nc.sy.*, nucleus of syncytium; *pr.*, prestereomal area; *pl.*, peritoneum; *rg.mb.*, regenerated membrane; *sp.*, spicule; *st.*, stereom; *sy.*, syncytium; *trab.*, trabecula.

through the body wall of *S. drobachiensis* which had been decalcified and stained the following relations of the stereom and the surrounding tissues may be observed. The epidermis (Fig. 23) appears on the outer surface of the section and beneath this there is a thin layer of syncytial reticular connective tissue (*pr.*) which is directly continuous on its inner surface with the definitive stereom (*st.*). This syncytium is loosely organized and has large intersyncytial spaces within which are found wandering "amœbocytes with spherules." The cytoplasm of the syncytium is fibrous in appearance and the remnants of spicules could be observed in it. The traces of spicules were more marked in the region bordering on the stereom where the syncytium had the trabecular organization characteristic of the stereom. This region I have designated as the prestereomal area. The stereom is made up of I-shaped trabeculæ which are apparently joined to each other end to end, so that the whole stereom is a framework of beams and rafters with regular lacunar spaces. The substance of the stereom when observed in the unstained condition is clear crystalline in character and when stained is intensely basophilic in reaction. A prestereomal area is also found between the stereom and the peritoneum. From the organization and relation of the prestereomal areas to the stereom it is evident that the growth of the stereom takes place peripherally by the gradual deposition of skeletal material within the prestereomal trabeculæ. Nuclei with prominent nucleoli were observed in the prestereomal trabeculæ, but none were observed in the trabeculæ of the stereom. This condition would indicate that trophic activity of the cells is lost in giving rise to the stereom and that the whole cytoplasm of the syncytium becomes converted over into skeletal material while the nucleus degenerates. Now if a cut were made directly through the body wall and a piece of it removed, the cut surface would present three regions, a middle stereom region and two peripheral prestereomal areas. It is obvious that the prestereomal areas would be capable of replacing certain parts of the test, but the question arises as to whether or not these cells are aided in this regeneration by the leucocytes.

Although the body wall of several specimens of *S. drobachiensis* were resected in an attempt to answer this question, the results are far from convincing and the description of the regeneration of the test which follows is to be studied more in detail at a later date.

Eight specimens of *S. drobachiensis* were injected with carmine in seawater through a minute perforation in the peristomial membrane and were allowed to remain in a live box for twenty-four hours, so that the leucocytes would have a chance to take up the carmine particles. At the end of this time, a piece of the body wall, 1 cm. square, was removed from the aboral surface of each specimen. The resected specimens were then put into a live box and observations were made over a period of four weeks upon the changes which were taking place in the resected area. When first observed it was noted that a membrane was gradually closing the opening in the body wall. This membrane was reddish in color as contrasted with the greenish color of the surrounding body wall, and it grew mesially from the margin of the opening so that the latter diminished slowly in diameter. In seven of the eight specimens the opening in the body wall was closed in two weeks. At first the membrane closing the opening was very delicate, but it gradually became firmer and in several specimens toughened. This phase of the toughening of the membrane occurred during the third week. It was then noticed that skeletal material began encroaching on the margin of the membrane which was contiguous with the original cut surface. During the fourth week the deposition of skeletal material went further and several individuals showed a portion of regenerated test. At this time the tissue closing the opening was removed from several specimens, spread out on a slide and studied in the fresh condition. The whole mass had a crimson color and was of a tangled fibrous consistency in which were apparent leucocytes containing carmine granules which had formed more or less of a syncytium, in which the cell boundaries were indistinct. Several pieces of the membrane and the adjacent body wall were preserved for sectioning in order to determine the relation of the cells to the replacement of the skeleton. These pieces were decalcified and a series of sections made.

Figure 23 is a semi-diagrammatic drawing of a vertical section through the regenerated membrane and a portion of the adjoining body wall, the structure of the latter having been discussed above. In the membrane there are several regions of differentiation. The region most distal to the test and forming the center of the membrane is thick and reddish in color, this color being due to the presence of minute particles of carmine in the cells which make up the syncytial reticulum of the membrane

(Fig. 24). The color diminishes laterally and also in the region proximal to the stereom, where the syncytium is trabeculated in the same manner as it is in the prestereomal peripheral areas (Fig. 25). Since the leucocytes were the only cells in the perivisceral fluid which were observed to be phagocytic and form syncytia it is probable that they aid the prestereomal cells in the formation of the membrane and gradually develop skeletal material for the formation of the stereom. The cytoplasm of the reticulum in the membrane in addition to the carmine particles contains fibers which are probably the remnants of decalcified spicules. The nuclei of the reticulum are round, have a distinct nucleolus and are the same type as are present in free leucocytes observed in the lacunæ of the stereom and in the prestereomal trabeculæ, so that it is probable that the leucocytes and the connective tissue cells of the prestereomal area are of the same series, except that one has become specialized for the production of the stereom under ordinary conditions of growth, whereas the leucocytes only take over this function when the body wall is injured. The only evidence for a line of demarcation between the two is in the presence of the carmine particles in those cells which make up the reticulum of the membrane. It is therefore evident that the membrane is formed by both the multiplication of the prestereomal connective tissue cells aided by the anastomosis and syncytial formation of the leucocytes which make up the bulk of the membrane. Within the spaces of the reticulum of the membrane are found large numbers of "amœbocytes with spherules" which probably carry nutrition to the cells of the syncytium, enabling them to carry out their scleroblastic function. These "amœbocytes with spherules" are very few in the region of trabecular formation adjacent to the stereom and are entirely absent from the lacunæ of the stereom, thus they seem to be massed in that region where repair is going on rapidly and the cells of which are being differentiated.

Thus in brief there are three regions present in the regenerating area. First, the syncytial region which forms the bulk of the membrane which has closed the opening in the body wall and is composed of a syncytium of leucocytes with small lacunæ, containing large numbers of "amœbocytes with spherules." Secondly a prestereomal area, definitely trabeculated, with large lacunæ and continuous with the peripheral prestereomal areas which enclose the stereom of the adjacent region of the body wall. The "amœbocytes with spherules" are very few in this region. Thirdly, the definitive regenerated stereom which is crystalline in character, devoid of nuclei and in the lacunæ of which there are no "amœbocytes with spherules."

It is obvious that the cellular elements which form the membrane have either been derived directly from cells in the region of the prestereomal areas or from cells of the perivisceral fluid which have migrated to this area and formed syncytia. Since the leucocytes are the only cells of the perivisceral fluid which have been observed to form syncytia and since they are the cœnocytes of the original wandering mesenchymal cells, there is reason to suppose that they have some part in this process. That stereom formation does not necessarily start from the cut surface of the resected area is evidenced by the appearance of independent areas of stereom formation in the membrane (Fig. 23). A more detailed study of the relationships of the leucocytes to the regeneration of the test is to be more closely followed in a more extended series of experiments.

These observations agree in general with Théel's conclusions that the scleroblastic cells "ought to be enrolled among the true plasma-amœbocytes, though they are dissimilar as to their functional manifestations, and consequently, too, as to their chemical constitution. It is not unlikely that their quality of taking up a superfluity of inorganic salts from the surrounding medium has influenced the cells, their power of amœboid movement having been suppressed or limited." He concludes that the directions of the pseudopodia predetermine the characteristic protrusions of the crystals in *Psolus phantapus*. The crystal when formed occupies the whole of the cell except for a small space occupied by the nucleus. In the formation of the stereom in *S. drobachiensis* a similar predetermination of the organization of the skeletal elements is apparent in the prestereomal areas.

From the above observations it may be concluded that the leucocytes in both the active and passive phases are those cells which are of constant occurrence in the Echinoderms. They are phagocytic, thrombocytic and possibly scleroblastic. These cells are omnipresent in all regions of the perivisceral cavity.

From their activities they must be recognized as the most generalized cells in the perivisceral fluid since no others have been observed whose functions are so diversified. The question of their origin is a vexing one, although it was once pointed out that the dorsal organ may, in the Asteroidea, be an organ of leucoblastic function, a function which Cuenot ('01) denies this organ, claiming that it is more likely an organ for the elimination of wornout cells of the perivisceral fluid and that the "amœbocytes" (general term) are probably peritoneal in origin and may also arise from each other. In all probability the number of leucocytes increases rapidly when an animal is injured, particularly in the region of the injury, where they form the framework for the regeneration of other tissues. They may be regarded as one of the important agents in the replacement of lost parts in the Echinoderms.

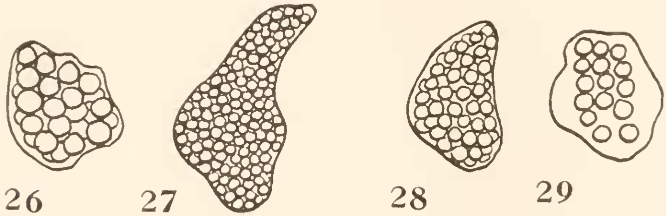


FIG. 26. "Amœbocyte with red spherules," *Strongylocentrotus drobachiensis*, cam. luc., × 1300.

FIG. 27. "Amœbocyte with red spherules," *S. franciscanus*, cam. luc., × 1300.

FIG. 28. "Amœbocyte with red spherules," *Echinarachnius eccentricus*, cam. luc., × 1300.

FIG. 29. "Amœbocyte with yellow spherules," *E. eccentricus*, cam. luc., × 1300.

2. *The "Amœbocytes with Spherules."*

There are two groups of "amœbocytes with spherules" present in the perivisceral fluid of the Echinoderms. One group of these is characterized by the presence of pigmented spherules in the cytoplasm and the other by colorless spherules. In both types the spherules fill the cytoplasm to such an extent that the nucleus appears merely as a light space in the center of the cell. All of the "amœbocytes with spherules" are further characterized by the presence of very blunt pseudopodia and when fluid containing them is allowed to stand they tend to assume a spherical shape.

Amœbocytes containing red spherules were observed in the

Echinoidea alone, those containing yellow spherules in the Echinoidea and some of the Holothuroidea, and those containing colorless spherules in the Ophiuroidea, Echinoidea and Holothuroidea.

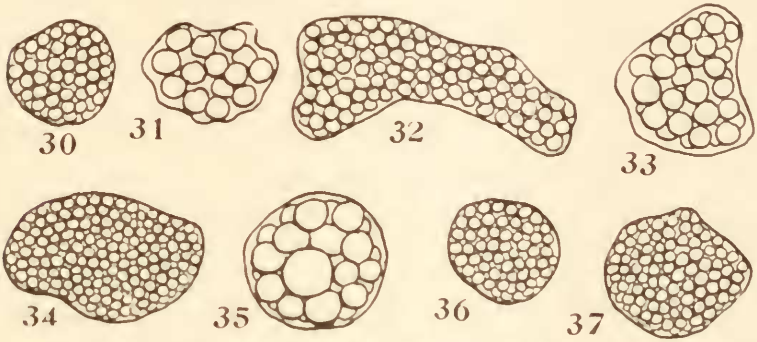
In the Echinoidea, the "amœbocytes with red spherules" were most numerous and largest (12 microns in diameter) in *S. franciscanus* (Fig. 27); in *S. drobachiensis* (Fig. 26) they were less numerous and smaller (9 microns in diameter). In *Echinarachnius eccentricus* (Fig. 28) they were also less numerous and smaller (10 microns in diameter). The predominant type of pigmented amœbocyte in *E. eccentricus* was the type with yellow spherules (Fig. 29) and these were particularly massed on the peritoneum of the intestine. Scattered yellow pigmented amœbocytes were observed in *Stichopus californicus*, but no association with any organ system was noted.

The red pigment in the pigmented amœbocytes of the Echinoidea was designated echinochrome by McMunn ('85). The view of this author and Griffiths ('87) that the amœbocytes containing echinochrome are associated with oxygen transportation has never been fully accepted. Cuenot ('91b) was the first to oppose this assumption and stated that there was no change in the depth of the color when the cells were allowed to stand in the air, and that the contained pigment instead of being an oxygen-carrying pigment was stored-up food material which the cells had taken from the intestine. Further, Winterstein showed that a solution of echinochrome does not take up more oxygen than the same amount of seawater. This assertion of Winterstein's is significant, because it opens up the question as to the oxygen requirements of the Echinoderms. Of course it is obvious that all of the free oxygen used by the Echinoderms is taken from the seawater and further it has been shown that except for a difference in albuminoid content the perivisceral fluid of the Echinoderms is the same density as the seawater (Cuenot, '91). Therefore, we can assume that there is a diffusion of the seawater through the breathing organs of the Echinoderms and that the oxygen content is the same in the perivisceral fluid as it is in the outside seawater. With the exception of certain of the Holothuroidea, all of the Echinoderms have a very slight development of rapidly contractile muscle elements and hence no need for a large amount of oxygen for muscular activity, from which it follows that in the Asteroidea, Ophiuroidea, Echinoidea and Holothuroidea with tests (*e.g.*, *Psolus*), which live in regions of high free oxygen content, due to tidal currents, there is enough oxygen in suspension in the perivisceral fluid for ordinary metabolic activity. Therefore in these forms no oxygen-carrying cells are developed and the cellular modifications which occur in the cells of the perivisceral fluid may be regarded as independent of the relations of the breathing organs. Further we would expect to find efficient oxygen-carrying cells in the Holothuroidea which have a relatively high development of muscle, for the needs of which the free oxygen content of the perivisceral fluid is not sufficient. The hemocytes are the cells which fulfill this requirement in the Holothuroidea and will be discussed later.

If the "amœbocytes with red spherules" are not to be regarded as oxygen-carrying cells, are they then related to the transfer and storage of food as suggested by Cuenot? As a partial answer to this query are the results and conclusions of Awerinzew ('11) who carried on investigations on the habitat and food relations of two varieties of *S. drobachiensis*. Awerinzew observed that the two colored varieties of this species lived in different types of environment, the green-yellow forms living on a mud and stone bottom and the red forms amongst red algæ. He assumed that the pigment in the food of the red forms was carried from the intestine to the skin and deposited there. The pigment in the perivisceral cells would then be due to the food eaten. He checked his results by injecting carmine particles in solution into the alimentary tract and found that these particles were carried to the skin, but the distinctions between the types of cells engaged in this activity were not made clear, so that it is possible that the cells were leucocytes carrying on their normal activity as phagocytes and there is no case for the pigmented cells as food carriers.

The "amœbocytes with red spherules" are far more numerous and larger in the perivisceral fluid of *S. franciscanus* than they are in *S. drobachiensis*. This fact leads to the suggestion that the color of the body wall is due to a difference in numbers of the red cells in the two species.

Since it has not been proven that the "amœbocytes with red spherules" are developed from other cells by the ingestion of pigment from food, it may be that they are the descendants of the pigmented cells (chromatophores) of the larval Echinoidea. These cells are present in the segmentation cavity of the larval Echinoids, but I have not found any reference as to their occurrence in the larvæ of other classes. If this is true, then we are dealing with an amœbocyte which is specific in the Echinoidea and may yet be found to be derived from the colored substances characteristic of the Echinoid ovum.



FIGS. 30–37. "Amœbocytes with colorless spherules," cam. luc., × 1300. 30. *Ophiopholis aculeata.* 31. *S. drobachiensis.* 32. *S. franciscanus.* 33. *E. eccentricus.* 34. *C. japonica.* 35. *Stichopus californicus.* 36. *S. californicus.* 37. *C. chronjhelmii.*

"Amœbocytes with colorless spherules" (Figs. 30–37) are more widely distributed in the Echinoderms than the pigmented ones and are found in the Ophiuroidea, Echinoidea and Holothuroidea. The cells of this type are much more abundant and active in the Holothuroidea than in the other classes. They are also much less stable and disintegrate soon after withdrawal from the body. They are deeply stained by methylene blue-seawater or neutral red-seawater solutions, a reaction which is not characteristic of the "amœbocytes with colorless spherules" in the other classes.

It is to be noted that the size of the spherules is constant for any given amœbocyte, but that there is a variation in the sizes of the spherules of amœbocytes characteristic of different animals. Théel has also remarked upon this and has pointed out that the "amœbocytes with colorless spherules" predominate in the Holothuroidea which he studied. He says: "In the holothurids the white corpuscles are thoroughly predominant, though, there also, species are to be met with which are characterized by both white and red corpuscles. Thus, for inst., *Labidoplax buskii* makes an exception by lodging cells with hyaline granules together with such ones which contain red pigmented granules, both kinds being nearly equal in number."

The origin and functions of the "amœbocytes with colorless spherules" is problematical. According to Cuenot ('91b) they may arise from leucocytes by the addition of spherules of protein and are hence to be regarded as food carriers.

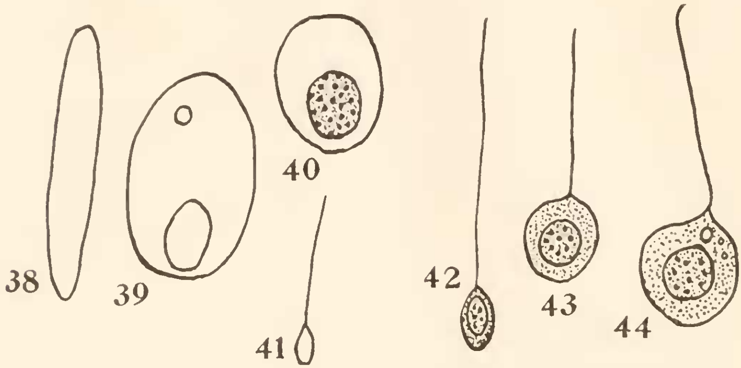


FIG. 38. Hemocyte, *C. japonica*. Side view, cam. luc., × 1300.
FIG. 39. Hemocyte, *C. japonica*. Surface view, cam. luc., × 1300.
FIG. 40. Hemocyte, *C. chronjhelmii*. Surface view, cam. luc., × 1300.
FIG. 41. Vibratile corpuscle, *Stichopus californicus*, cam. luc., × 1300.
FIG. 42. Vibratile corpuscle, *Ophiopholis aculeata*, cam. luc., × 1300.
FIG. 43. Vibratile corpuscle, *Strongylocentrotus drobachiensis*, cam. luc., × 1300.
FIG. 44. Vibratile corpuscle, *S. franciscanus*, cam. luc., × 1300.

3. *The Hemocytes.*

Théel ('21) describes a series of "red blood corpuscles" from the perivisceral fluid of the Holothuroidea, excepting the forms with tests, as *Psolus*. In his discussion of these cells, he calls attention to the fact that the content of these corpuscles was first noted by Semper ('68) who suggested that this material was of the nature of hemoglobin. Howell ('85) advanced the same suggestion with regard to the same type of cell in certain species of *Thyone* and *Cucumaria*. Cells of this type were also observed in the Holothuroidea by Cuenot ('91b), Knoll ('93) and Kollman ('08). These cells were described as spherical or elongate ovoid cells with a definite limiting membrane which was elastic, but did not form pseudopodia. The cytoplasm was of a homogeneous color of the same shade as hemoglobin, and in it was embedded a slightly ovoid nucleus.

In my observations on the genera *Cucumaria* and *Stichopus* I have found these cells to be limited to the *Cucumaria*. For reasons given below I have designated these cells as hemocytes. The hemocytes of *Cucumaria* are flattened, biconvex discs, ovoid in shape. The cell membrane is plastic, but the cells exhibit no amœboid movement and are carried passively in the perivisceral fluid by contractions of the vasa and movements of the body. The cytoplasm is a homogeneous mass of orange-yellow color in which is located a small oval nucleus, eccentrically placed (Figs. 38, 39, 40). In *C. chronjhelmii* the granular content of the nucleus is very clearly apparent (Fig. 40). A mass of these cells presents a crimson appearance comparable to the oxygenated blood of vertebrates. The hemocytes of *C. japonica* (red body wall) are much larger and more numerous than those of *C. chronjhelmii* (white body wall), consequently the deep color of the former may be due in part to this difference in number.

Van der Heyde ('22), although giving no reference to the earlier suggestions that these cells contain hemoglobin, carried on a series of experiments on the content of hemocytes in *Thyone briareus*. He subjected a solution obtained from these cells to the spectroscope and obtained a band characteristic of oxyhemoglobin; upon reduction the solution gave the single band characteristic of hemoglobin and when shaken with air, the double band characteristic of oxyhemoglobin appeared; hemin-like crystals were obtained from the content of the hemocytes. These experiments together with several other chemical tests have led Van der Heyde to conclude that the substance is hemoglobin. As a result of these observations I have designated these cells, types of which also occur in *Cucumaria*, the hemocytes, a term which is briefer and more concise than the appellation red blood cells.

We may ask if there is any reason why oxygen-carrying cells should be present in certain of the Holothuroidea and not in other members of this class or other Echinoderm classes. I have suggested earlier in this paper that oxygen-carrying cells may be regarded as unnecessary in the Asteroidea, Ophiuroidea and Echinoidea because of the relatively low oxygen needs of the body which can be supplied by free oxygen of the perivisceral fluid. But in the Holothuroidea of the types like *Cucumaria* and *Thyone*, the body is made up of highly contractile muscle elements which may possibly need more oxygen than can readily be supplied by the perivisceral fluid. Consequently, it is suggested that the hemocytes, as oxygen carriers, have appeared in association with the development of the muscular system and greater muscular activity, so that the contractile elements may be supplied. Further evidence for this suggestion is found in the absence of these hemocytes in the Holothuroidea without muscular bodies (*e.g.*, *Psolus*) which are comparable to the test-bodied Echinoidea.

4. *The Vibratile Corpuscles.*

The vibratile corpuscles are those cells in the perivisceral fluid which are minute and bear flagella. Cells of this type were observed in *Ophiopholis aculeata* (Fig. 42), *S. drobachiensis* (Fig. 43), *S. franciscanus* (Fig. 44) and *Stichopus californicus* (Fig. 41).

In *O. aculeata* the vibratile corpuscle is very minute (3 microns in diameter). A small peripheral layer of granular cytoplasm encloses a relatively large nucleus. A single long flagellum is present at one end of the cell. The vibratile corpuscles of *Strongylocentrotus* are much larger, each has a relatively smaller nucleus as compared with the amount of cytoplasm and a shorter flagellum than the vibratile corpuscle of *O. aculeata*.

In *S. californicus*, the vibratile corpuscles are very minute (1 micron in diameter) and are colored with a yellowish pigment which obscures the nucleus, if such be present. Since hemocytes are lacking in this species it may be assumed that these cells are oxygen-carrying cells and that the pigment is a hemin compound related to the content of the hemocytes of the other muscular-bodied Holothuroidea.

Cuenot ('02) suggested that the function of the vibratile corpuscles is to keep the fluid content of the perivisceral cavity in motion, thus aiding the ciliated peritoneum in causing a circulation of the fluid.

III. Summary.

From a comparative study of the cellular elements in the perivisceral fluid of a representative group of Echinoderms found in the vicinity of the Puget Sound Biological Station the following conclusions have been reached:

1. The leucocytes are constant in the perivisceral fluid of the Echinoderms.

2. The leucocytes in all of the Echinoderms studied are phagocytic and thromboblastic and in some species appear to be scleroblastic and associated with the replacement of resected skeletal areas.

3. Hemocytes (cells with hemoglobin) are found only in certain of the Holothuroidea and are correlated with the development of a highly muscular body.

4. Modifications of the breathing organs have apparently no effect on the cellular contents of the perivisceral fluid, so that no specific oxygen-carrying cells are developed in those forms with a rigid non-muscular body wall despite the limitations of the breathing organs.

5. Of the "amœbocytes with spherules," those with colorless spherules are present in the Ophiuroidea, Echinoidea and Holothuroidea and are predominant in the last class. "Amœbocytes with red spherules" are present in the Echinoidea, where the size and number present is correlated with the depth of color of the body wall.

6. Vibratile corpuscles are present in the Ophiuroidea, Echinoidea and in *Stichopus californicus* alone of the Holothuroidea studied. In the latter species they are pigmented and are regarded as cells which function as do the hemocytes in other muscular-bodied Holothuroidea.

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